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Hormonal and Type-Dependent Adhesion of Group B Streptococci to Human Vaginal Cells

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Adhesion of group B streptococci to epithelial cells of the human vagina proved to be type dependent and to fluctuate during the menstrual cycle with a maximum near the time of ovulation. Oral contraception completely abolished the observed cyclic changes. Reduced serum levels of luteinizing hormone (less than 5 mIU/ml) and of follicle-stimulating hormone (less than 10 mIU/ml) were associated with a 10-fold reduction in adhesion of B streptococci to vaginal cells.

In recent years group B streptococci have been increasingly recognized as the causative agent of serious neonatal infections. Whether this is due to a real increase in incidence or to improved isolation techniques is unknown (1). Studies from various countries relate the newborn infections with maternal genital tract colonization and the transmission of the microorganism from the mother to the offspring during delivery (4-6, 12).

It has already been reported that vaginal colonization among nonpregnant women does not differ significantly from that observed among parturients. An increased rate of isolation during the middle of the menstrual cycle has been reported, and, though some interesting hypotheses have been proposed (3), factors influencing the carrier state have not yet been determined.

Since adhesion to epithelial cells can be considered the first step in host-bacteria interactions (7) leading to colonization and finally to overt infection, group B streptococci adhesion to vaginal epithelial cells has been investigated to verify a possible dependence of the adhesion ability on hormone-mediated changes in this ecosystem. A previously described in vitro system (8) has been utilized which avoids possible interfering conditions that may exist in vivo. Although this experimental approach may be criticized as being different from the in vivo situation, it is suitable and widely employed (7, 9-11) for understanding the properties of the microorganism under investigation.

The strains used throughout the study were in part isolated from pathological material examined in our clinical bacteriology department and in part kindly provided by A. M. Molina, Institute of Microbiology, University of Siena, Italy. Strains were routinely grown on blood agar base medium enriched with 8% sheep blood or in Todd-Hewitt broth. Grouping was performed by the Lancefield acid extraction method using commercially available antisera (Behringwerke, A. G., Marbur-Lahn, West Germany). Serotyping was performed at the *Staphylococcus* and *Streptococcus* Unit, Center for Disease Control, Atlanta, Ga., and in our laboratory. A total of 68 strains was tested; 14 belonged to type Ia, 12 to type Ib, 14 to type II, and 20 to type III.

Two groups of healthy volunteers were followed for 4 months. One group of 10 women used oral contraception (norgestrel, 0.25 mg; ethinyl-estradiol, 0.05 mg), and the other group of 10 did not (control group). All of them remained free from infections for the period of the study, and none became pregnant. Cells were obtained by scraping twice the mucosal surface of the vagina with an Ayre spatula. Cells obtained from the first sample were discarded. To test adhesion of the various strains, cells were always taken in the same week of the menstrual cycle from women belonging to the control group. To test adhesion during the menstrual cycle, cells were obtained from the two groups of volunteers twice a week, and data from the same week were plotted together.

Vaginal cells, immediately after sampling, were washed four times by centrifugation (300 rpm, 15 min) in phosphate (50 mM)-buffered saline (pH 7.2 or 5.4), and the final pellet was suspended in 0.5 ml of the same buffer. Cell number was determined by a Thoma counting chamber.

Bacteria from 48-h-old cultures in Todd-Hewitt broth were washed twice in phosphate-

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buffered saline at neutral or acidic pH and suspended in a final volume to yield approximately 2×10^8 bacteria per ml. Equal volumes of the bacterial suspension and the cell suspension were mixed to obtain a ratio of 1,000:1. Incubation was carried out at 35°C for 40 min with periodic stirring. After this time interval, the vaginal cells were washed four times by differential centrifugation to discard nonadhering bacteria. The pellet was stained with Gram or May-Grünwald stain, and a slide stained by the Papanicolau procedure was also prepared. The number of adhering bacteria per cell was counted by direct microscopic examination of 200 cells.

A significant difference in adhesion ability was observed among the four serotypes tested (Table 1). Type III strains showed the highest ability to attach to vaginal cells; Ia and II serotypes possessed an intermediate degree of adhesiveness; and the Ib serotype had a very poor capability to adhere to epithelial surfaces. This behavior seemed to be type dependent, since a similar pattern was observed when adhesion of the same strains to oral epithelial cells was tested. From Table 1 it is also evident that the adherence of all serotypes was greater at neutral pH than at acidic pH. Adhesion of type III streptococci was least affected by a low pH.

To examine whether any change in cell receptiveness occurred during the menstrual cycle, 20 strains (5 type Ia, 3 type Ib, 4 type II, and 8 type III) were tested for adhesion with cells taken from the two groups during each week of the cycle.

A fluctuation in cell receptiveness was detected in the 10 women who were not using oral contraceptives. It followed the same pattern for all the streptococci tested, and only data for type III will be reported here (Table 2). A progressive increase in receptiveness was evident in all cases; the maximum was reached around day 14 after menses. Most frequently, the highest number of bacteria adhering per cell occurred during the third week, but in three subjects (as in C) it consistently occurred earlier. In the week preceding menstruation a sudden fall in receptiveness was the common feature in all the subjects examined. The differences in cell receptiveness observed during the weeks of the cycle were exposed to statistical evaluation by using Student's t test and were proven to be highly significant ($P < 0.001$).

Oral contraception completely abolished the cyclical changes such that no increase in cell receptiveness occurred during the midcycle period. Two examples of this peculiar pattern, from the 10 volunteers examined, are presented in Table 2. Interestingly enough, a patient with amenorrhea associated with low serum levels of luteinizing hormone (<5 mIU/ml) and follicle-stimulating hormone (<10 mIU/ml) had a very low level of receptiveness (34 bacteria adhered per cell) with absolutely no variations for the entire 4-month period (data not shown).

These data clearly demonstrate that group B streptococci type III possess the greatest ability to adhere to vaginal cells and are less affected by acidic pH. Thus the data obtained in vitro are consistent with clinical observations indicating that type III is either the most common isolate or possesses a striking degree of virulence (2, 10). One would be more likely to isolate from a vaginal swab type III strains which, because of the acidic pH, would overwhelm the colonization by the other, more sensitive, serotypes. On the other hand, a sample from cervical mucus, which is neutral or slightly basic, would probably yield an increased rate of isolation of serotypes other than III. The frequency of serotypes isolated from cervical and vaginal swabs was reported in a carefully documented study (12). It was stated

TABLE 1. *Ability of group B streptococci to adhere to vaginal cells, related to serotype and pH of incubation*

Type	No. of strains tested	Bacteria adhered per cell at: pH 7.2 Range	pH 7.2 Mean	pH 5.4 Range	pH 5.4 Mean	Difference
Ia	14	35–408	153	8–276	65	$P < 0.001$
Ib	12	0–71	12	0–8	3	$P < 0.001$
II	14	16–364	60	4–118	25	$P < 0.001$
III	20	42–653	249	16–501	176	$P < 0.005$

TABLE 2. *Group B streptococci adhered per cell during the menstrual cycle*

Subject	Streptococci adhering during week[a]: 1	2	3	4	Difference: week 1/week 2
Control group (10 volunteers)					
Subject A	104	343	379	219	$P < 0.001$
Subject B	62	93	102	71	
Subject C	84	252	244	107	
Contraceptive users (10 volunteers)					
Subject K	123	134	138	136	NS[b]
Subject L	94	90	94	92	
Subject M	90	88	90	92	

[a] Each value represents the mean of eight determinations (twice a week for four cycles).

[b] Difference not significant.

that on 24 cervical specimens Ia serotypes accounted for 25% of isolates, whereas type III strains comprised 16% only. In vaginal cultures the situation was completely reversed: type III strains accounted for 30% of total isolates, whereas types Ia and Ib were, respectively, 15.7 and 9.5% of total.

The observed changes in cell receptiveness during the menstrual cycle seem primarily due to modifications in the properties of the cell surface, since from the morphological appearance of the cells it was easy to exclude the possibility that cells in different maturation stages (i.e., basal or parabasal) were being tested. Moreover, they are independent of the bacterial strain used or of other factors (such as saprophytic flora, menstrual blood, iron, or secretions) that have been excluded by the experimental conditions employed.

It has recently been reported by Ofek and colleagues (10, 11) that *Escherichia coli* binds to mannose residues present on the surface of the epithelial cells. It seems reasonable to suggest a similar mechanism for group B streptococci, and, although their cellular receptor(s) are at present unknown, our experiments suggest that their synthesis or accessibility is dependent on hormonal control.

The results obtained with women using contraception have to be considered with a note of caution. Further studies, including other bacteria, are required to clarify the effect of oral contraception on colonization by genital tract pathogens.

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